GENOMICS INFORMATICS PROTEOMICS METABOLOMICS

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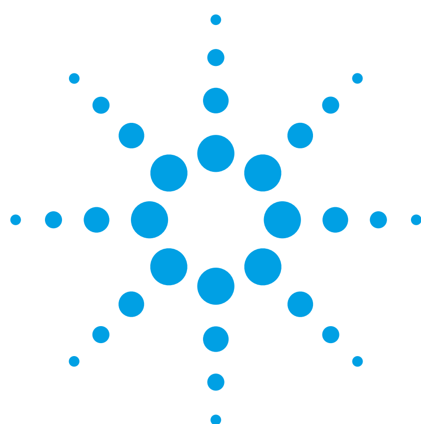

New Stringent Two-Color Gene Expression Workflow Enables More Accurate and Reproducible Microarray Data

Authors

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Abstract

Agilent Technologies continues to forge ahead in the development and optimization of robust microarray processing protocols. New product lines are being developed to optimize processes associated with microarray analysis such as sample labeling, hybridization, wash and data analysis. This study compared the new Agilent Two-Color Microarray-Based Gene Expression Analysis (Version 4.0) protocol with the Legacy Agilent 60-mer Oligo Microarray SureHyb Enabled/SSPE Wash (Version 2.1, April 2004) protocol. Agilent has developed this higher stringency protocol as part of their dual-mode gene expression system that uses 60-mer oligonucleotide microarrays for either one-color or two-color analysis. The system can be run in conjunction with recent versions of Agilent's Feature Extraction software (Version 8.1 and higher) to generate a quality control (QC) report for each microarray. The QC report includes a performance characterization of the new Agilent RNA Spike-In control targets. To compare the two protocols, changes in gene expression between two human RNA samples were analyzed on Agilent microarrays using either the new Version 4.0 or the older Version 2.1 platform. The protocols differed with respect to the composition and temperature of the hybridization and wash buffers. Data presented demonstrate that Agilent's Gene Expression Version 4.0 protocol generates microarray data with lower system noise and more accurate log ratios, ensuring greater reproducibility. The improvements observed for the microarray data were validated with QuantiGene (Genospectra, Inc), a non-microarray gene expression assay that directly measures mRNA using cooperative hybridization and branched DNA technology.

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Introduction

Improving parallel gene expression data reproducibility, accuracy, and sensitivity is critical to success in microarray analysis. All components of the microarray workflow (such as probe design, printing process, RNA sample quality, labeling, microarray processing, scanning, and feature extraction algorithms) contribute to quality research results. Optimized hybridization and wash conditions result in heightened sensitivity, while simultaneously improving the accuracy of gene expression ratios and consistency of data. The higher stringency Version 4.0 protocol is part of a novel gene expression package, merging new and existing components of the Agilent microarray platform. The optimization of the Two-Color Gene Expression protocol has paved the way for development of Agilent's One-Color Gene Expression Platform. This study compares the performance of Agilent's Legacy/SSPE (Version 2.1) protocol with that of the innovative Two-Color Gene Expression (Version 4.0) protocol for processing 60-mer microarrays.

The Version 2.1 and Version 4.0 protocols differ in several ways, including addition of "spike-in" controls to the labeling reactions, alterations in microarray hybridization, wash procedures and Feature Extraction (FE) software algorithms. Improvements in accuracy based on the use of Agilent's exogenous RNA spike-ins were validated using the QuantiGene gene expression assay, which targets a dozen unique, non-control genes. The QuantiGene assay measures mRNA directly to determine gene expression in a given sample. This is accomplished by amplification of the mRNA signal via cooperative hybridization and branched DNA technology, resulting in sensitive and accurate measurements.

Materials and Methods

Microarrays

Agilent 60-mer microarrays printed with SurePrint™ technology were used for this study, based on the Agilent Human 1A (V2) Gene Expression microarray design (part number G4110B). This microarray design allows detection of over 18,000 well characterized, full length human genes using one specific probe for each sequence. These microarrays also contain ten replicated probes (over-sampled probes) for 100 selected genes that were used to compute the intra-array reproducibility.

RNA Labeling

Poly A+ RNA samples from human adult heart (AH) and human fetal heart (FH) (Clontech, Mountain View, CA, product numbers: 636113 and 636156) were labeled with the Agilent Low Input Linear Amplification Kit (part number 5184-3523) as per the recommended protocol. One microgram of each poly A+ RNA was labeled in the presence of cyanine 3-CTP or cyanine 5-CTP (Perkin Elmer/NEN, product numbers NEL 580 and NEL 581) in separate labeling reactions. Multiple labeling reactions were performed and the labeled cRNA targets were pooled and stored at -80°C.

The same pooled cRNA targets were used in all hybridizations. Agilent RNA Spike-In Mix (part number 5188-5279) was added to the mRNA samples prior to the labeling reactions following the RNA Spike-In Kit protocol. The Agilent RNA spike-in control targets are a set of ten *in vitro*-synthesized polyadenylated transcripts, derived from the adenovirus E1A gene. Each transcript was premixed into two different spike cocktails, at known concentrations. The ten transcripts were present in mass equivalents spanning a 200-fold dynamic range representing ratios from 0.1 to 10 (see

Table 1. Final Relative Sample Concentrations of the RNA Spike-Ins

RNA Spike-in name	Cy3-A relative copy number	Cy5-B relative copy number	Expected Relative Ratio
(+)E1A_r60_1	10	10	1:1
(+)E1A_r60_n11	1.5	0.5	3:1
(+)E1A_r60_a20	100	100	1:1
(+)E1A_r60_3	3	9	1:3
(+)E1A_r60_a104	10	30	1:3
(+)E1A_r60_a107	30	10	3:1
(+)E1A_r60_a135	9	3	3:1
(+)E1A_r60_a22	10	100	1:10
(+)E1A_r60_a97	0.5	1.5	1:3
(+)E1A_r60_n9	100	10	10:1

Table 1 for details). The spike-in control targets were used to monitor both the labeling reactions and the microarray performance.

Microarray Hybridization

Microarray hybridizations were carried out in Agilent's SureHyb Hybridization Chambers (part number G2534A) containing 750 ng of cyanine 3-labeled and 750 ng of cyanine 5-labeled cRNA per hybridization. Color swaps were performed as follows: four replicate hybridization reactions with cyanine 3-FH versus cyanine 5-AH (designated as polarity +1) and four replicate color swap reactions with cyanine 3-AH versus cyanine 5-FH (designated as polarity -1), resulting in a total of 16 microarray hybridizations to test both protocols.

The hybridization reactions with the Legacy/SSPE Version 2.1 protocol were performed at 60˚C for 17 hours using Agilent's In Situ Hybridization Kit Plus (part number 5184-3568), following procedures as described in the Agilent 60-mer Oligo Microarray SureHyb Enabled/SSPE Wash (Version 2.1, April 2004) protocol. The In Situ Hybridization Kit Plus includes a tube of 10x control targets. The 10x control targets in this kit are formulated with both the blocking agents and a TAMRA-labeled control oligonucleotide that hybridizes to the positive control features on the microarrays.

The hybridization reactions with the higher stringency Version 4.0 protocol were performed at 65˚C for 17 hours using Agilent's Gene Expression Hybridization Kit (part number 5188-5242), following procedures as described in the Agilent Two-Color Microarray Based Gene Expression Analysis (Version 4.0, December 2005, publication number G4140-90050) protocol. The Gene Expression Hybridization Kit includes a tube of 10x blocking agent that is no longer formulated with the TAMRA-labeled control oligonucleotide.

Washing

With the Legacy/SSPE Version 2.1 protocol, the hybridized microarray slides were disassembled and washed for 1 minute at room temperature in SSPE wash solution 1 (6x SSPE + 0.005% Sarcosine). After the initial washing, the slides were placed in SSPE wash solution 2 (0.06x SSPE + 0.005% Sarcosine) for one minute at room temperature. The microarrays were then treated with Agilent Stabilization and Drying Solution (part number 5185-5979) at room temperature for 30 seconds.

Following the stringent Version 4.0 protocol, the hybridized microarrays were disassembled at room temperature in Agilent Gene Expression Wash Buffer 1 (part number 5188-5325). After the disassembly, the microarrays were washed in Gene Expression Wash Buffer 1 for one minute at room temperature, followed by washing with Gene Expression Wash Buffer 2 (part number 5188-5326) for one minute at an elevated temperature (approximately 31˚C). The microarrays were then treated with Agilent Stabilization and Drying Solution at room temperature for 30 seconds. The optional use of the Agilent Stabilization and Drying Solution protects the cyanine signal from degradation in laboratories with high levels (>50 ppb) of ozone or other airborne oxidants.

Scanning, Feature Extraction and Data Analysis

Microarrays were scanned using the Agilent DNA Microarray Scanner (part number G2565BA). The microarray images generated by the scanner were analyzed with Agilent Feature Extraction Software (Version 8.5, part number G4148AA) using the GE2_22K_1205 FE extraction protocol. The Feature Extraction files were imported into Microsoft Access, Microsoft Excel, and Spotfire DecisionSite for further data analysis. T-tests comparing the two protocols were performed on filtered data to omit results that were either below acceptable detection levels ("IsPosAndSig" flag = false), saturated ("IsSaturated" flag = true), or uneven ("IsFeatNonUnifOL" flag = true) in either the green or the red channels. The Total Access statistics program was used to perform the t-test.

Microarray Data Validation Using the QuantiGene Assay

The QuantiGene assay (Genospectra, Fremont, CA, product number QG0003) is a non-array gene expression assay that uses branched DNA technology to amplify the signal of a highly specific hybridization event, rather than amplifying the RNA target. Signal amplification is precisely controlled by the QuantiGene probe set design and is more reproducible than either target amplification or PCR. The basis of the improved specificity is the cooperative hybridization of mRNA to the probe set and capture plate. The final luminescent readout is directly proportional to mRNA quantity and is not altered by processes like RNA purification or target amplification. The same aliquots of human adult heart and human fetal heart Poly A^+ RNA used in the microarray experiments were tested with the QuantiGene assay.

Table 2. Components and Procedure Comparisons of Legacy/SSPE Version 2.1 and Version 4.0 Protocols

Components	SSPE/Legacy	Version 4.0
Spike in controls	None*	RNA Spike-In Kit (5188-5279)
Labeling	Low Input Linear Amplification Kit (5184-3523) Fluorescent Direct Labeling Kit (G2557A) **	Low Input Linear Amplification Kit (5184-3523)
Hybridization	In Situ Hybridization Kit Plus (5184-3568) 60˚C for 17 hours	Gene Expression Hybridization Kit (5188-5242) 65˚C for 17 hours
Wash	Homemade SSPE Wash Buffers Disassemble: 6 x SSPE + 0.005% Sarcosine at room temperature Wash 1: 6 x SSPE + 0.005% Sarcosine at room temperature Wash 2: 0.06 x SSPE 0.005% Sarcosine at room temperature Wash 3: Stabilization and Drying Solution at room temperature (5185-5979) at elevated temperature	Agilent Gene Expression Wash Pack (5188-5327) Disassemble: Agilent Gene Expression Wash Buffer 1 at room temperature Low Stringency Wash: Agilent Gene Expression Wash Buffer 1 at room temperature High Stringency Wash: Agilent Gene Expression Wash Buffer 2 S&D Wash: Stabilization and Drying Solution at room temperature
Scan	DNA Microarray Scanner BA (G2565BA)	DNA Microarray Scanner BA (G2565BA)
Feature Extraction	FE 7.5 (G2567AA)***	FE 8.5 (G4148AA)

* In this study the exogenous spike-in control targets were added to RNA samples prior to all of the labeling reactions and were used with the Legacy/SSPE Version 2.1 protocol to highlight differences between the old and new protocols.

** The Legacy/SSPE Version 2.1 protocol uses either the amplification kit or the direct labeling kit. The Version 4.0 protocol only supports the use of amplified cRNA targets.

*** Feature Extraction Version 8.5 with the GE2_22K_1205 FE protocol was used to extract all of the microarrays in this experiment (both the Legacy/SSPE Version 2.1 and the Version 4.0 microarrays) in order to generate the new Agilent microarray Quality Control reports and the associated data to enable direct comparison between the two protocols.

Component Comparison of Old and New Protocols

Table 2 summarizes the major similarities and differences between the Legacy/SSPE (Version 2.1) and Two-Color Gene Expression (Version 4.0) protocols, including reagents and processing steps.

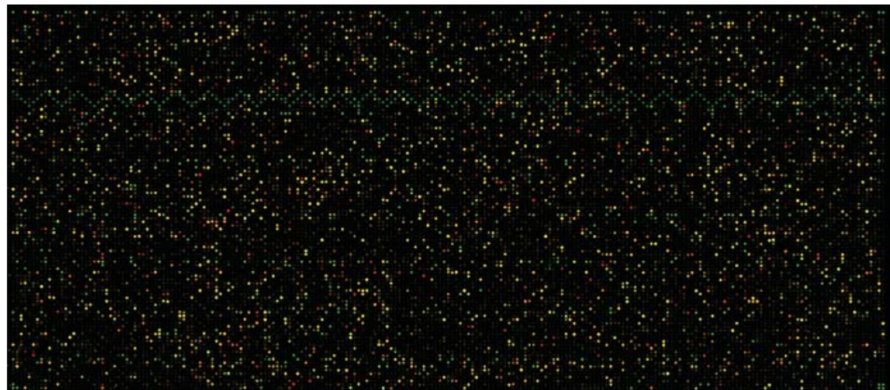

Figure 1A.

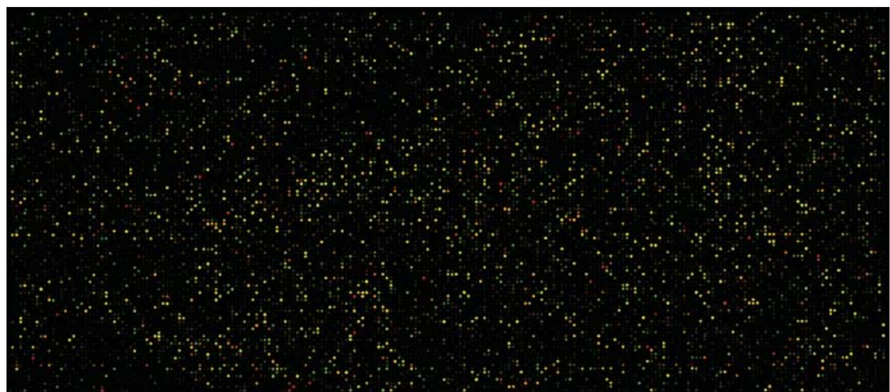

Figure 1B.

Figure 1. Microarray image generated by Legacy/SSPE (Version 2.1) and Two-Color Gene Expression protocols (Version 4.0). Figure 1A. The image produced by the Version 2.1 protocol has additional green features due to the addition of TAMRA-labeled control targets to the hybridization mix. These control targets generate green signals for the positive control probes, which include the corner probes. **Figure 1B.** In the image generated by the Version 4.0 protocol, the corners do not light up because the TAMRA labeled control targets are no longer part of the control targets in the new Gene Expression Hybridization Kit.

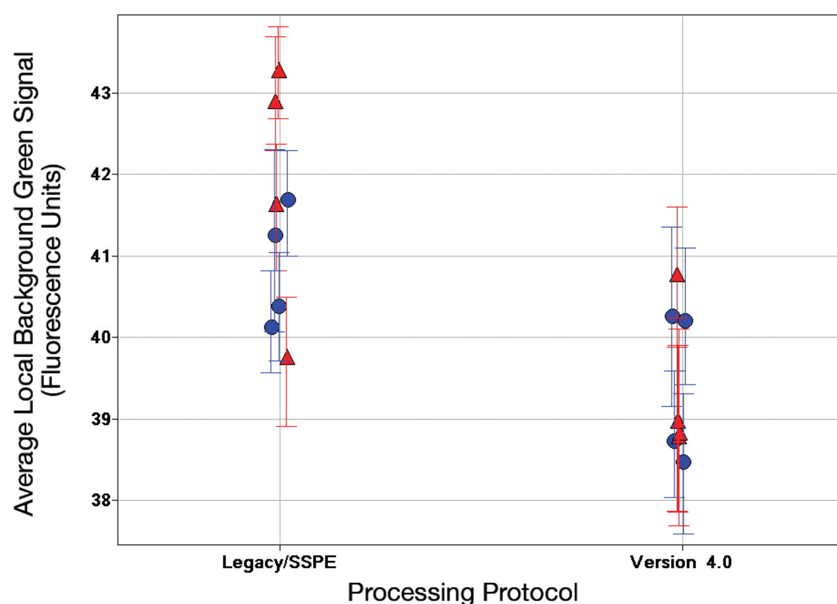

Figure 2A.

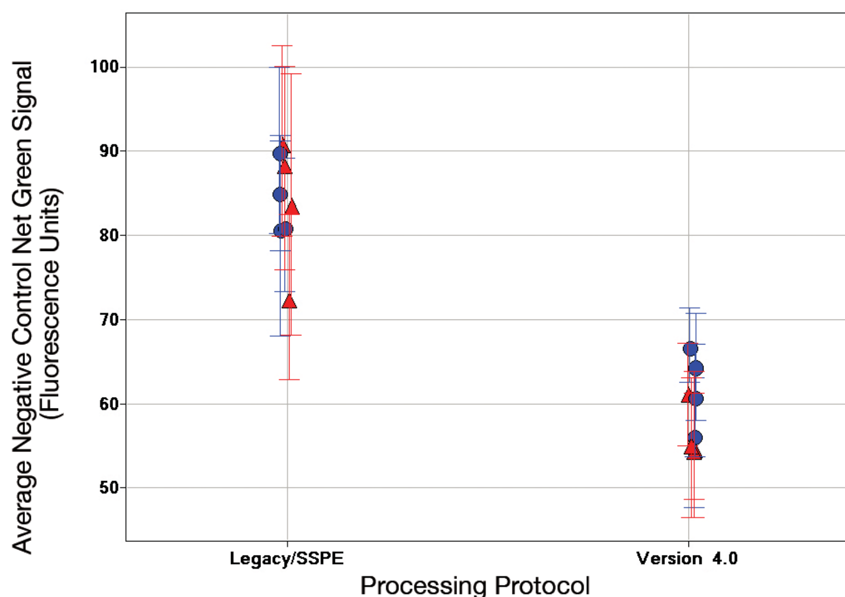

Figure 2B.

Figure 2. Averages of Local Background and Negative Control Signals per Microarray. In both figures, the X-axis represents the two different processing protocols with the Legacy/SSPE Version 2.1 protocol in the left column and the Version 4.0 protocol in the right column. In **Figure 2A** the Y-axis represents the average of the local background raw green signal (before the scanner offset subtraction). In **Figure 2B** the Y-axis represents the average of the negative control net green signal. Net signals have the scanner offset subtracted from the raw scanner signals. The average scanner offset was 24 counts for this experiment. Each spot represents the average green signals of one individual microarray, with error bars representing standard deviation. The spots are shaped and colored by polarity: Red Triangle: polarity -1 (cyanine 3-AH versus cyanine 5-FH); Blue Circle: polarity +1 (cyanine 3-FH versus cyanine 5-AH).

Results and Discussion

The microarray experiments were designed to compare the performance of the Legacy/SSPE Version 2.1 and new Version 4.0 protocols. The experiments addressed microarray data questions of sensitivity, reproducibility, system noise, and accuracy of log ratios.

Microarray Images

The images generated by the Agilent microarray scanner (.tif) for the two protocols are similar with the exception of additional green TAMRA-labeled controls accompanying the Legacy/SSPE Version 2.1 protocol.

The 10x control targets containing TAMRA-labeled oligos were necessary for earlier versions of Feature Extraction software (Version 7.5 and older) to place a grid on the microarray images. Improvements in the feature finding algorithms with newer versions of the software do not rely on green corners for grid placement, thus eliminating the need for TAMRA-labeled controls. Microarray signal fidelity was improved by removing non-specific cross-hybridization of the TAMRA-labeled controls with non-control probes in the Version 4.0 protocol.

System Noise

Lower background equates to reduced noise in microarray data. Significant reductions in microarray local backgrounds were obtained with the new Version 4.0 protocol as demonstrated in Figure 2A.

Figure 2A illustrates the degree of local background reduction using the Version 4.0 protocol (approximately 30% lower). The reduction in local background was also correlated to the improved performance of the negative

control probes. Figure 2B demonstrates a 30% reduction in the green net signals of the negative control probes. The microarrays processed with the Version 4.0 protocol also generated more consistent net signals for the negative controls with smaller standard deviations across the eight microarrays illustrating how a reduction in system noise can substantially increase system reproducibility.

Reproducibility

One measure of system reproducibility is the variability between replicated samples, both within a microarray and across replicates. Agilent offers 100 non-control probes, replicated 10 times on each microarray that can be used to measure intra-array reproducibility. The signals from the 10 replicate probes were averaged and the standard deviation was used to determine the coefficient of variation (CV) between the replicate probes of different microarrays. The CV value was multiplied by 100 to generate a percent coefficient of variation (%CV). The Feature Extraction program calculates and ranks the %CV for each of the non-flagged, non-control "oversampled" genes. The median %CV of the non-control genes were reported on the QC Reports and compared across individual microarrays as shown in Figure 3.

When averaged, the median %CV across eight microarrays was very similar for the new Version 4.0 protocol (9.47%) as compared to the SSPE/Legacy Version 2.1 protocol (9.31%). However, the distribution of the CVs among the eight microarrays was much tighter for the Version 4.0 protocol (Figure 4).

The Version 4.0 protocol had significantly lower average %CV's (15.4%) as compared

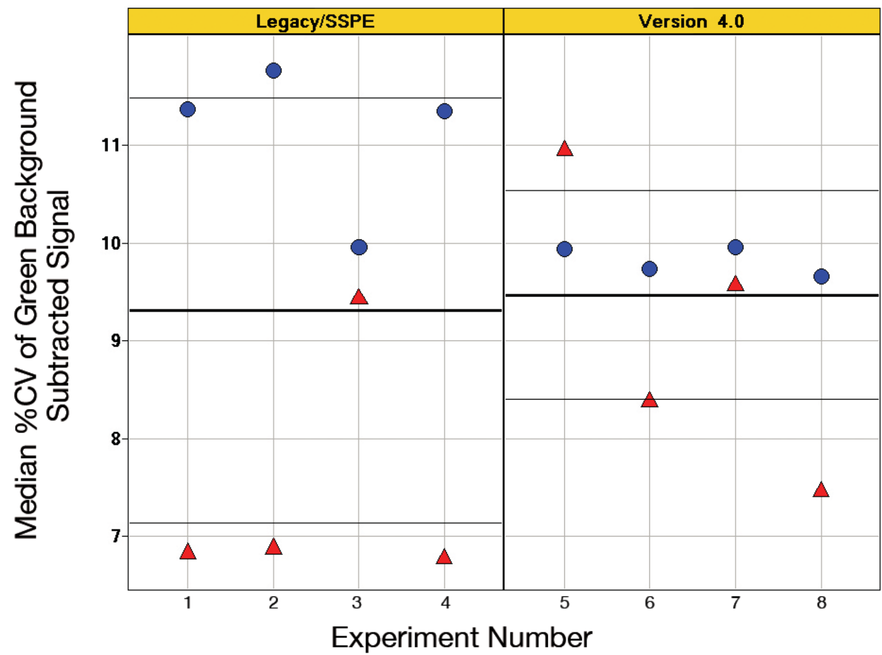

Figure 3. Intra-array Median %CV of the Non-Control Green Background Subtracted Signals Each spot represents the median %CV of one individual microarray experiment. The spots are shaped and colored by polarity: Red Triangle: polarity -1; Blue Circle: polarity +1. These values were obtained from the Feature Extraction QC Report. Each vertical line represents a microarray experiment comprised of a dye-swap pair (both + and - polarities). Experiments 1-4 were replicate microarrays processed with the Legacy/SSPE Version 2.1 Protocol, while experiments 5-8 were replicates processed with the Version 4.0 Protocol.

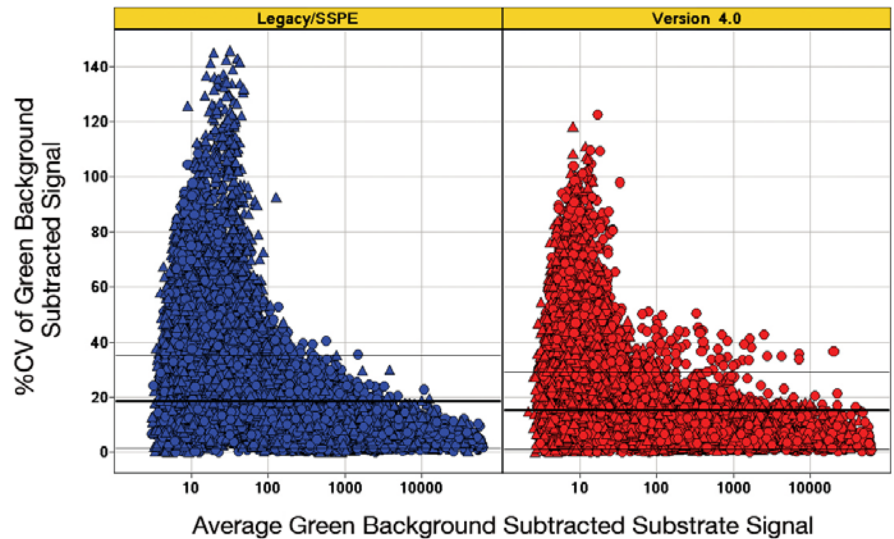

Figure 4. Inter-array %CV of Background Subtracted Signals of Non-Control Probes. The X-axis represents the average green background subtracted signals at the individual feature level across 4 replicate microarrays and the Y-axis represents the %CV of the average green background subtracted signals across the same 4 microarrays. The blue spots indicate microarrays processed with the Legacy/SSPE Version 2.1 protocol and the red spots indicate microarrays processed with the Version 4.0 protocol. The spots are shaped by polarity where the triangles = polarity -1 and circles = polarity +1.

to the Legacy/SSPE Version 2.1 protocol (18.6%). The difference in %CV was impressive considering that lower signals tend to have a higher CV and the Version 4.0 protocol generated a lower overall signal than the Legacy/SSPE Version 2.1 (Figure 7). Again, the data using the Version 4.0 protocol (in red) was tighter and lower than that generated with the Version 2.1 protocol (in blue).

Accuracy

The accuracy of microarray results can be validated using a number of different methods, including addition of control transcripts at known ratios or comparison of gene expression ratios with other non-microarray technologies. Log ratio compression has long been a concern in microarray data analysis. This experiment shows that use of the Version 4.0 protocol resulted in a significant reduction in log ratio compression, generating data with higher accuracy as confirmed by E1A Spike-In (Figure 5) and QuantiGene data (Figure 6). The main contributor to the improvements in log ratios is believed to be the increased hybridization temperature utilized by the Version 4.0 protocol.

When expected log ratios for Agilent's Spike-In transcript (see Table 1 for details) were compared to the observed ratios, the Version 4.0 protocol appeared to be superior, once again. A slope of 1.0 reflects agreement between the microarray data and the expected log ratios based on the formulation of the Agilent Spike-In Kit. The Legacy/SSPE Version 2.1 protocol had a slope of 0.879, indicating that 87.9% of expected log ratios were observed (Figure 5A). In contrast, the Version 4.0 protocol had a slope of 0.933 (93%, Figure 5B),

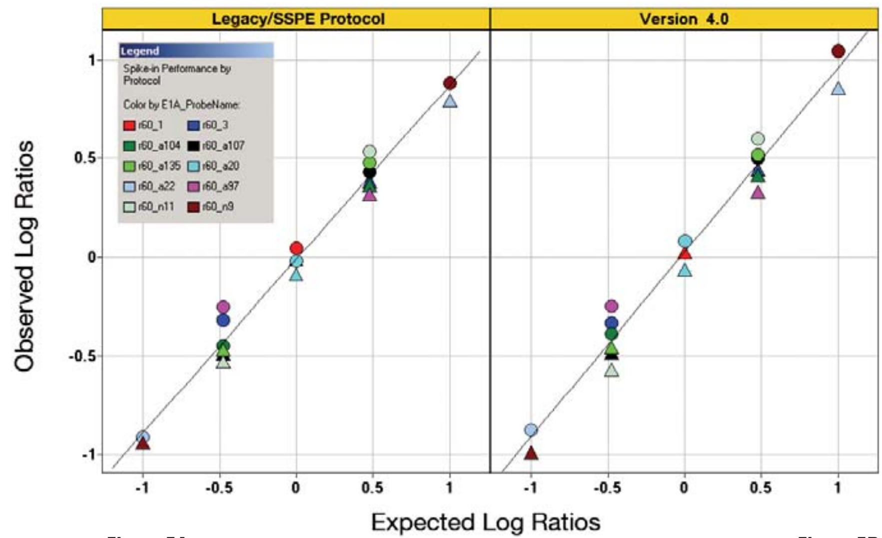

Figure 5A. Figure 5B.

Figure 5. Comparisons of Expected Log Ratios Versus Observed Log Ratios of the E1A Spike-In Probes. The X-axis represents the expected log ratios based on the amount spiked into either the adult or the fetal heart RNA. The Y-axis represents the microarray mean log ratios for each E1A gene across the four replicate microarrays for each polarity. These spots can represent the average of up to 120 features (30 replicate probes per microarray times 4 microarrays) and are colored by E1A probe name and shaped by polarity: triangle = polarity -1, circle = polarity +1. **Figure 5A:** Legacy/SSPE Version 2.1 protocol. **Figure 5B:** Version 4.0 protocol. The Agilent QC Report generates log ratio accuracy data similar to Figure 5 for each microarray.

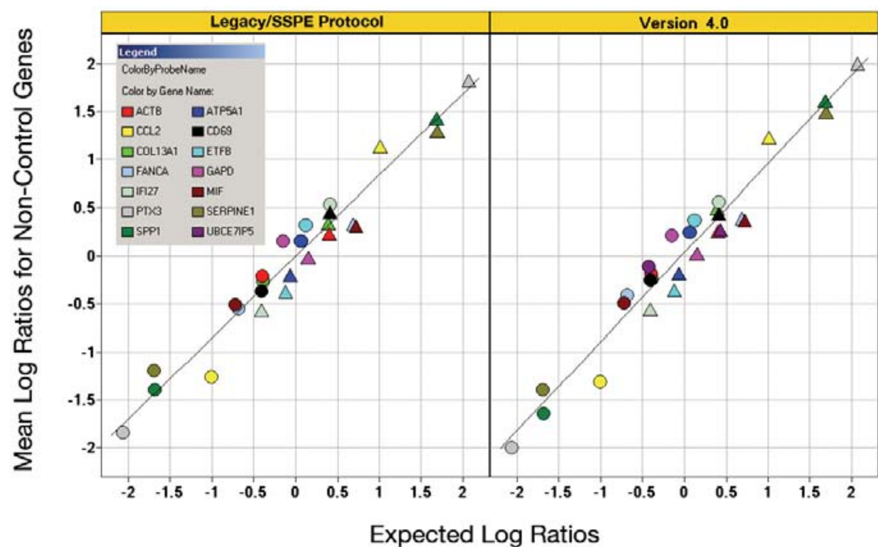

Figure 6A. Figure 6B.

Figure 6. Linear Regression Analysis of QuantiGene Probes. The X-axis represents the expected log ratios based on the QuantiGene assay. The Y-axis represents the microarray mean log ratios for each non-control gene across the four replicate microarrays for each polarity. These spots represent the average of up to 40 features (10 replicate probes per microarray times 4 microarrays) and are colored by gene name and shaped by polarity where a triangle = polarity -1 and a circle = polarity +1. **Figure 6A:** Legacy/SSPE Version 2.1 protocol. **Figure 6B:** Version 4.0 protocol.

demonstrating that a higher percentage of expected log ratios were observed (in essence, decompressed log ratios). The correlation coefficients were high for both protocols.

The gene expression levels for a number of unique, non-control genes were validated using the orthogonal QuantiGene assay. Upon comparing the expected log ratios for specific genes with the QuantiGene assay and the observed ratios for Agilent microarray results, once again the Version 4.0 protocol generated less compressed log ratios (Figure 6).

While the Legacy/SSPE Version 2.1 protocol had log ratios that were 84.7% of expected values, the Version 4.0 protocol had log ratios that were 92.6%. The increase in accuracy for log ratios using the QuantiGene assay was similar to that observed for E1A Spike-In transcripts, demonstrating that the biological and Agilent Spike-In positive control probes behaved similarly in both workflows. The decompression of observed log ratios (Figures 5 and 6) indicate that the Version 4.0 protocol provides more accurate microarray data.

Sensitivity

The sensitivity of a microarray system is determined by combined efficacy of low expression gene detection and the ability to detect differentially expressed genes with low fold changes between RNA samples. Signal intensities for approximately half of the microarray features remained the same using the Version 4.0 protocol and of those that differed, most exhibited a lower signal. Results indicated that the increased stringency of the new Version 4.0 protocol reduced non-homologous

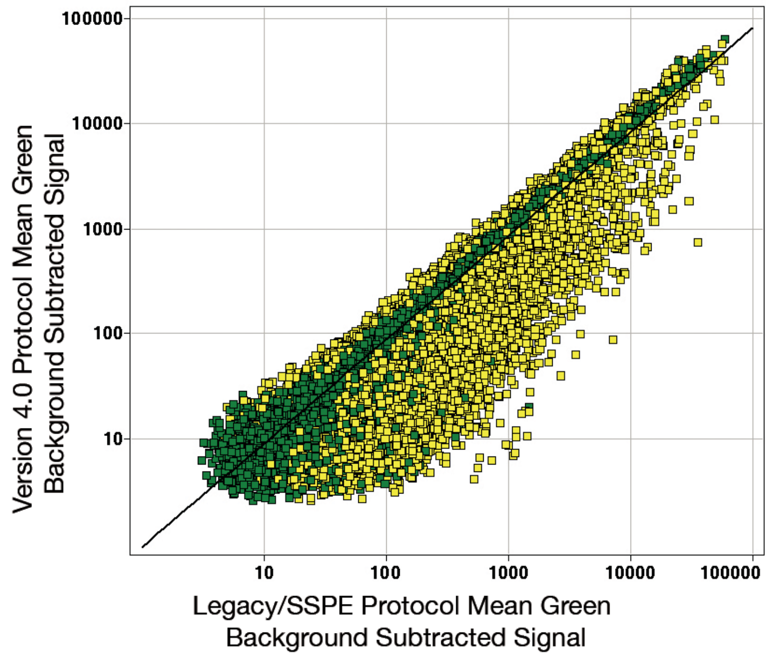

Figure 7. T-test of Green Background Subtracted Signal Intensity of Non-Control Probes. The X-axis represents the green background subtracted signal intensities of probes with the Legacy/SSPE Version 2.1 protocol. The Y-axis represents the green background subtracted signal intensities of the probes with Version 4.0 protocol. Points are colored green when they are statistically the same ($p>0.01$) between the two protocols and colored yellow when they are considered to be statistically different.

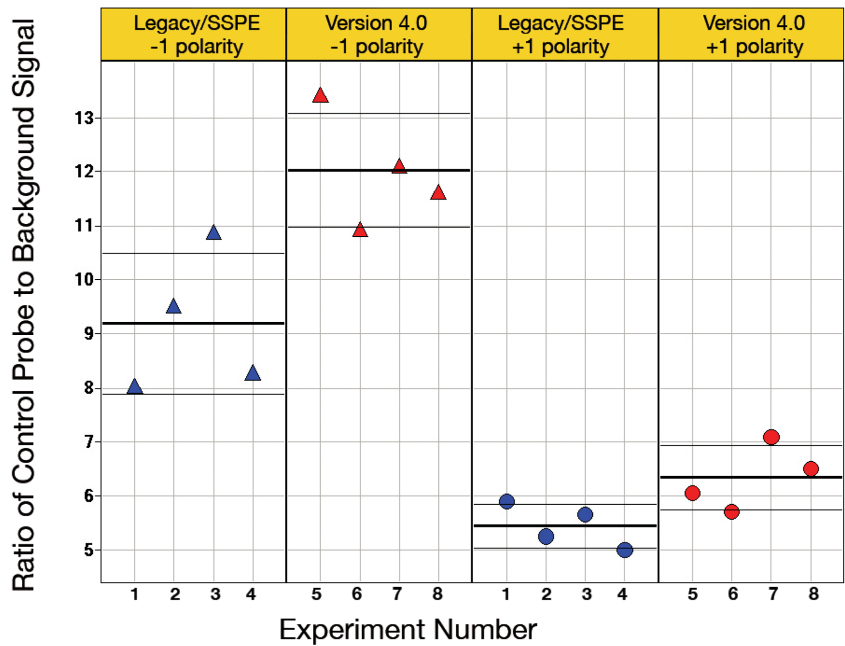

Figure 8. Ratio of Spike-In Control E1A_r60_n11 to the Background Signal. The expected relative copy number of the E1A r60_n11 probe in the -1 polarity is 1.5 copies per cell and the expected relative copy number in the +1 polarity is 0.5 copies per cell. The data is grouped and shaped with the -1 polarity (triangles) in the two columns on the left and the +1 polarity (circles) in the two columns on the right. The spots are colored by protocol where blue = Legacy/SSPE Version 2.1 protocol and red = Version 4.0 protocol.

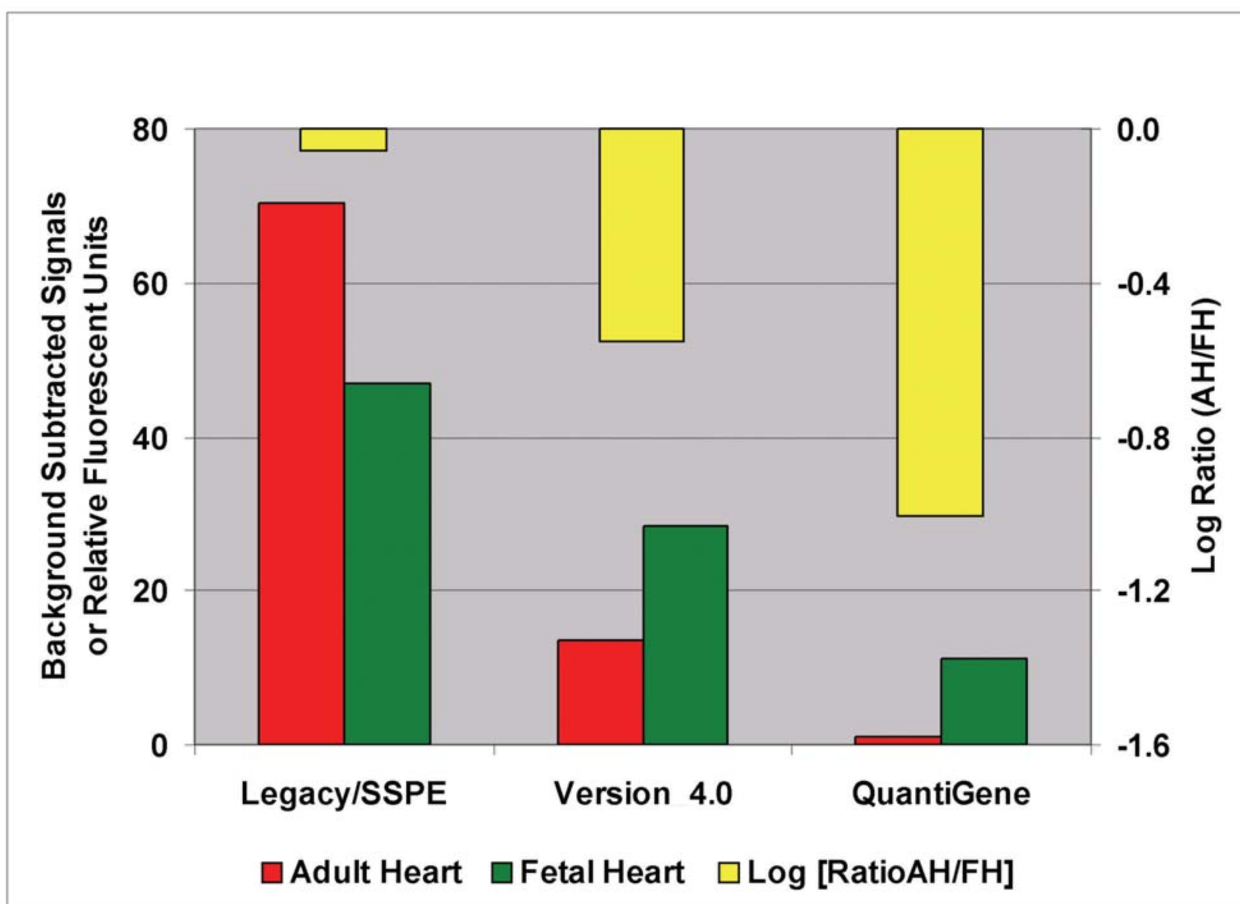

Figure 9. Gene Expression Comparison of the Abundance Gene CCNA2. The X-axis represents the method used to determine the expression of the CCNA2 gene (probe name: A_23_P58321). The primary Y-axis represents either the red or green background subtracted signals in the +1 polarity for the Agilent microarrays or the relative fluorescent units for the QuantiGene assay. The secondary Y-axis is the log ratio of the adult heart to the fetal heart as determined by either the Agilent Feature Extraction software or as calculated for the QuantiGene data.

cross-hybridization, thus lowering the intensity of many signals (Figure 7).

Figure 7 demonstrates that over half (56.6%) of the microarray features were statistically significantly different between the two protocols based on statistical analysis ($p > 0.01$). Of the 56.6% that were different, 95% of those features have lower signals with the Version 4.0 protocol. Though decreased signal intensity is a cause for concern, further analysis indicated that lower signals did not equate to decreased sensitivity.

The sensitivity of each microarray experiment is reported on the Agilent QC Report and is defined as a ratio of the net signal of E1A spike-in probes with the lowest concentrations to the background signal (background used minus scanner offset), where a higher ratio correlates to higher sensitivity.

Both protocols were able to detect genes at low relative copy numbers (0.5 copies per cell in 1 million cells) with typical levels at least five times greater than the background (Figure 8). For both copy levels tested (0.5 and 1.5 copies per cell), the Version 4.0 protocol exhibited greater sensitivity than the Legacy/SSPE Version 2.1 protocol.

The detection of poorly expressed genes was also assessed using the QuantiGene assay. The QuantiGene assay exhibits increased sensitivity compared to Agilent microarrays due to elevated detection levels relative to background fluorescence. Thus, it may be able to detect genes at very low expression levels. The CCNA2 (Homo sapien cyclin A2) gene is an example of one low expressing, non-control gene. Agilent microarray signals were compared to QuantiGene for adult and

fetal heart RNA and the respective log ratios (Figure 9). The data represent averaged CCNA2 gene expression data from four replicate microarrays (+1 polarity) using either the Agilent microarray processing protocols or the QuantiGene assay.

Due to the considerable difference in fluorescence between the QuantiGene assay and the Agilent microarray (10,000-fold higher for QuantiGene), the QuantiGene relative fluorescent units (RFU) were divided by 10,000 to generate a reasonable working scale. This scale adjustment resulted in an average RFU of 1.1 for adult heart, 11.1 for fetal heart, and an average log ratio of -1.01. This log ratio calculation, based on the relative fluorescent units of the CCNA2 gene in the QuantiGene assay, resulted in a 10 fold expression difference for fetal heart mRNA as opposed to the adult heart. In the microarray experiment, the Legacy/SSPE Version 2.1 protocol generated an average red signal of 70.5, a green signal of 46.9, and an average log ratio of -0.055, suggesting no differential expression between the samples tested. The Version 4.0 protocol generated an average red signal of 13.6, a green signal of 28.3, and an average log ratio of -0.552. Although the Version 4.0 protocol exhibited significantly reduced red and green signals (relative to the Legacy/SSPE Version 2.1 protocol), the average log ratio was much closer to that of the orthogonal QuantiGene assay. Once again, the Version 4.0 protocol produced enhanced log ratios from lower signals.

Detection of Differential Expression

One benefit of Agilent's Feature Extraction software is the ability to

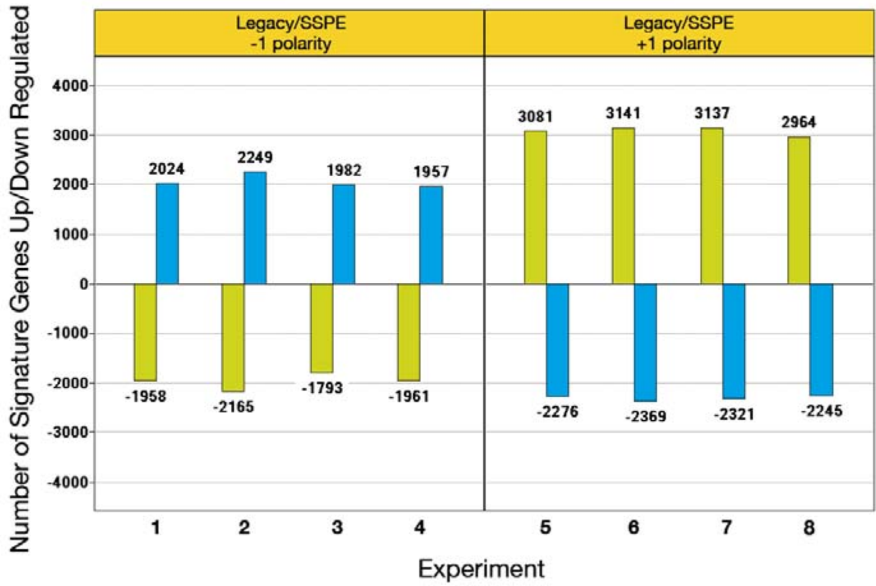

Figure 10A.

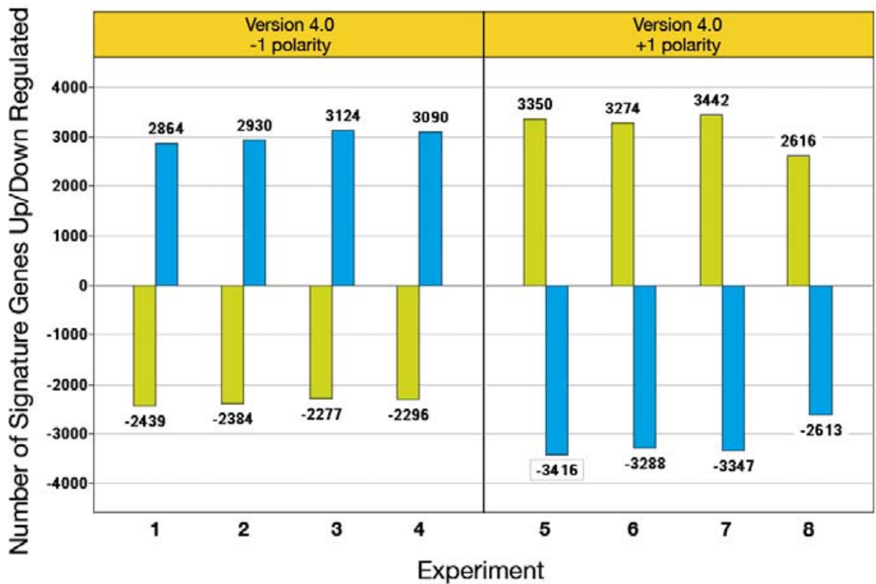

Figure 10B.

Figure 10. Count of Number of Differentially Expressed Non-Control Genes. The bars represent the number of up-regulated and down-regulated probes from replicate hybridizations for each dye swap polarity. Figure 10A is data from the Legacy/SSPE Version 2.1 protocol and Figure 10B is data from the new Version 4.0 protocol. The blue bars represent the number of up regulated probes in the -1 polarity and are colored the same as the number of down-regulated probes in the dye swapped +1 polarity. Conversely, the yellow bars represent the number of down regulated probes in the -1 polarity as well as the number of up-regulated probes in the dye swapped +1 polarity.

calculate the number of differentially expressed non-control probes on a given microarray. This study also compared the up- and down-regulated differential expression calls from the Agilent QC Report across the replicate microarrays (Figure 10).

A greater number of probes were considered to be differentially expressed with the Version 4.0 protocol (Figure 10B) as compared to the Legacy/SSPE Version 2.1 (Figure 10A). Use of the Version 4.0 protocol also increased the number of similar differential calls across replicate microarrays, giving researchers higher confidence in the accuracy of differential calls.

Conclusions

The new Version 4.0 Microarray protocol is a part of Agilent's dual-mode Gene Expression Microarray Platform. This integrated platform includes both the One-Color and Version 4.0 Platforms. The one-color and two-color detection options have a great deal of procedural overlap, including labeling and hybridization, scanner hardware, and data analysis software (Feature Extraction and GeneSpring GX). As compared to the Legacy/SSPE Version 2.1 protocol, the new Version 4.0 workflow provides microarray data with:

- Lower background intensities
- Reduced noise and fewer false positives
- Increased reproducibility
- Decompressed log ratios
- Heightened sensitivity of low expression level genes
- Robust workflow
- User-friendly tools for quality monitoring and troubleshooting

In conclusion, Agilent's new Version 4.0 Analysis System generates higher quality microarray data, subsequently boosting confidence in related results. The addition of the dual-mode platform allows researchers to choose the best detection options for their particular applications and experimental needs.

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Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's integrated biology solutions include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

For more information

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